

Fertility Preservation Methods in Breast Cancer

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Summary

Thanks to the recent advances in reproductive medicine, more and more young women with breast cancer may be offered the possibility of preserving their fertility. Fertility can be endangered by chemotherapy, by treatment duration and by patient's age at diagnosis. The currently available means to preserve a young woman's fertility are pharmacological protection with gonadotrophin-releasing hormone analogues during chemotherapy, and ovarian tissue or oocyte/embryo freezing before treatment. New future venues, including in vitro maturation, will improve the feasibility and efficacy of the fertility preservation methods in breast cancer patients.

Schlüsselwörter

Mammakarzinom · Chemotherapie ·
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Zusammenfassung

Dank der Fortschritte in der Reproduktionsmedizin kann jungen Frauen mit Brustkrebs ein Fertilitätserhalt angeboten werden. Die Fruchtbarkeit kann durch Chemotherapie, die Dauer der Behandlung und durch das Alter der Patientin bei Diagnose gefährdet sein. Die derzeit verfügbaren Methoden, die Fruchtbarkeit einer jungen Frau zu erhalten, umfassen den pharmakologischen Schutz mit Gonadotropin-releasing-hormone-Analoga während der Chemotherapie und Cryopreservation von Ovarialgewebe, Oozyten oder Embryonen vor der Therapie. Zukünftige neue Möglichkeiten, z.B. In-vitro-Reifung, werden die Durchführbarkeit und Effektivität des Fertilitätserhalts weiter verbessern.

Introduction

Breast cancer is the most common malignancy in women, and approximately 15% of breast cancers occur during the reproductive years of patients [1]. In the United States, 77,317 new cases of cancer in women aged 15–44 were diagnosed in 2008, with 9,542 in women aged 15–39 [2]. In this latter age group, the 5-year survival has increased from 75.2% in the 1970s to 86.9% today [2]. Given the relatively high incidence of breast cancer in women of reproductive age, the increased 5-year survival, and the rising trend of delaying pregnancy to later in

life, the issue of fertility preservation is gaining the attention of patients and the scientific community. The aim of this paper is to present an update of the fertility preservation methods available for breast cancer patients, which has been put together by physicians with different background and not necessarily involved in breast cancer care. Thus, this is not a comprehensive review of the published evidence regarding fertility preservation in breast cancer, but rather reports opinions related to the most up-to-date technologies available for assisted reproduction.

One of the main obstacles to fertility preservation in breast cancer is the lack of correct patient information and rapid referral to a reproductive endocrinologist. An American survey reported that only around 50% of cancer survivors recalled being counseled by a health provider on the impact of cancer treatments on fertility [3, 4]. The ASCO and ASRM guidelines suggest that oncology centers involved in breast cancer care should offer a thorough oncological/fertility counseling, where possible multidisciplinary by a clinical oncologist and a reproductive endocrinologist [5, 6]. The fertility unit must be able to manage these patients with minimal delay and offer the optimal option for each case [7].

Fertility Impairment in Breast Cancer Patients: What Matters?

Fertility can be impaired in breast cancer patients for a number of reasons: age at diagnosis, gonadotoxic chemotherapy, the duration of endocrine treatment (with the necessity to delay pregnancy desire or occurrence), or a combination of these factors.

Age at Diagnosis

Oocytes are progressively lost over the course of time from fetal life to menopause [8]. By the age of 37 years, around 290,000 of the 300,000 oocytes present at birth have already undergone apoptosis. The fecundity of a 40-year-old woman is halved compared to a 30-year-old companion, and the probability of a spontaneous abortion is tripled [9]. Most breast cancer patients in reproductive years are older than 35, and age per se represents an obstacle to optimal fertility and subsequent pregnancies in this patient population. As a basic element of counseling, the individual ovarian reserve should be assessed using an AMH (anti-Mullerian hormone) assay and/or transvaginal ultrasound examination with antral follicle count [10].

Chemotherapy-Induced Gonadotoxicity

Table 1 represents the degree of ovarian toxicity of specific drugs used for the treatment of cancer during reproductive years. The total dose is directly correlated to the ovarian dysfunction [11]. Age is also very important in changing the rates of chemotherapy-induced amenorrhea. Less than 5% of women aged 20–34 years had prolonged chemotherapy-induced amenorrhea, compared with 11% of women aged 35–39 and 40% of women aged 39 or older. The kind of chemotherapy is also important: women receiving doxorubicin/cyclophosphamide (AC) or AC plus paclitaxel were more likely to resume menses than those treated with AC plus docetaxel or cyclophosphamide/methotrexate/5-fluorouracil [12]. Table 2 summarizes the rate of chemotherapy-induced amenorrhea, segregated by different regimens used in breast cancer, according to the patients’ age.

The targets in chemotherapy-induced ovarian toxicity include primordial oocytes, granulosa cells and ovarian stroma [13]. Oktem et al. [14] reported apoptosis of primordial oocytes and follicles at 12 and 24 h after cyclophosphamide exposure, with a >90% reduction in follicle density at 48 h after treatment. Meirow et al. [15] reported significant vascular damage in histological sections of human ovaries exposed to chemotherapy. They described thickening and proliferation of cortical vessels, focal cortical fibrosis and segmental collagen deposition. Conversely, no ovarian toxicity has been reported for trastuzumab, a humanized IgG1 monoclonal antibody that recognizes the extracellular domain of c-erbB2 receptor [16].

The Tamoxifen Question

Few data are available about the direct ovarian toxicity of tamoxifen. Partridge et al. [17] reported lower AMH and higher FSH (follicle-stimulating hormone) levels in women who were still menstruating during tamoxifen treatment, but data are not conclusive. The main issue with tamoxifen is its prolonged treatment duration. The standard ‘20 mg/day for 5 years’ may significantly reduce the reproductive chances of a breast cancer patient diagnosed after 35 years of age, just for

High risk	cyclophosphamide, ifosfamide, busulfan, mechlorethamine, melphalan
Intermediate risk	doxorubicin, epirubicin, cisplatin, docetaxel, paclitaxel
Low risk	vincristine, methotrexate, 5-fluorouracil, trastuzumab

Table 1. Risk of ovarian toxicity of anti-neoplastic drugs

Regimen	Age < 30 years	Age 30–40 years	Age > 40 years
No treatment	1 %	< 5%	20–25%
AC ×4	–	13%	60%
CMF ×6	19%	35%	85%
CAF/CEF ×6	30%	–	85%
AC ×4 → Dtax	–	55%	–

Table 2. Rate of chemotherapy-induced amenorrhea, according to regimen and age

Modified from [63].
AC = Doxorubicin/cyclophosphamide, CMF = cyclophosphamide/methotrexate/5-fluorouracil, CAF = cyclophosphamide/ doxorubicin/5-fluorouracil, CEF = cyclophosphamide/epirubicin/5-fluorouracil, Dtax = docetaxel.

the physiological reduction of the oocyte quantity and quality that occurs with aging. Innovative protocols with shorter treatment duration are ongoing or have been proposed [18].

Fertility Preservation Methods

The main methods of fertility preservation in breast cancer patients are pharmacological protection of ovarian function during chemotherapy, ovarian tissue freezing, and oocyte harvesting after ovarian stimulation with subsequent oocyte or embryo freezing. The only non-experimental fertility preservation method, as reported by ASCO and ASRM guidelines [5, 6], is embryo cryopreservation. However, here we place more emphasis on newer techniques that are able to maintain patients' reproductive autonomy. In Italy embryo freezing is not allowed by the law, thus making alternative procedures a priority.

Pharmacological Protection of Chemotherapy-Induced Gonadotoxicity

Gonadotrophin-releasing hormone analogues (GnRHa) act by binding to the pituitary luteinizing hormone-releasing hormone (LHRH) receptors, resulting in their down-regulation with subsequent suppression of luteinizing hormone and estradiol production. This pharmacologically induced hypo-estrogenic milieu theoretically protects the ovaries from the gonadotoxicity of chemotherapy.

Several Phase II studies have reported a high rate of preserved menstruation in treated patients [19–22]. However, these studies were non-randomized, lacked a control group and involved small numbers of patients ($n = 24$ –100). Thus, large-scale randomized trials are needed. 4 such studies have been recently published. The first study included 78 patients randomized to standard chemotherapy with or without goserelin [23]. In this study, the goserelin group had a significantly lower incidence of premature ovarian failure (11 vs. 66%; $p < 0.001$), higher incidence of ovulation (69 vs. 25%; $p < 0.001$) and higher E2 levels (279 vs. 75; $p < 0.001$). The second study, the largest published, enrolled 281 patients, prospectively allocated to either chemotherapy alone or in combination with monthly triptorelin [24]. At an observation time of 12 months, the premature ovarian failure rate was 25.9% in the chemotherapy-alone group versus 8.9% in the chemotherapy + GnRHa group, an absolute difference of

–17% (95% CI: –26 to –7.9%; $p < 0.001$). The odds ratio for treatment-induced premature ovarian failure was 0.28 (95% CI: 0.14–0.59; $p < 0.001$). The third study enrolled 60 patients younger than 46 years of age with hormone-insensitive breast cancer [25]. Patients were allocated to receive chemotherapy with or without goserelin. Of the patients, 53 (88.3%) experienced temporary amenorrhea (93.3% with vs. 83.3% without goserelin). No significant difference was observed regarding the reappearance of menstruation at 6 months after chemotherapy (70.0% with vs. 56.7% without goserelin; difference of 13.3%; 95% CI, –10.85 to 37.45; $p = 0.284$). The fourth trial was planned for 124 patients with a scheduled 5-year follow-up, but it was stopped for futility after only 47 patients returned to follow-up. Menstruation resumed in 19 (90%) of 21 patients in the control group and in 23 (88%) of 26 in the triptorelin group ($p = 0.36$) [26].

The main limitation of these trials is that there are no data that confirm a significant increase of the ‘take-home’ baby rate. Only intermediate and loose indicators of fertility are considered: amenorrhea, biomarkers for ovarian reserve, and hormonal levels. While waiting for further studies, GnRHa administration should be considered experimental and be proposed in conjunction with other fertility preservation methods.

Ovarian Tissue Freezing

Less than 20 live births after autologous ovarian autografting have been reported until now. Table 3 reports a balanced compendium between advantages and risks of this procedure. The cryopreservation of ovarian tissue can be performed using fragments of ovarian cortex or the entire ovary. The whole ovary technique entails the potential advantage of ischemia prevention after transplantation, but has not been performed in humans and remains highly experimental [27, 28]. The most widely used technique in humans is the removal of ovarian cortical tissue samples. It is usually performed during conventional laparoscopy or by a robotic approach [29]. Primordial follicles from the human ovarian cortex survive in iced medium for about 4 h, even if other data demonstrate that transport can also take up to 20 h, without significantly affecting tissue characteristics [30]. Ovarian tissue cryopreservation can be performed using either the standard slow-freezing technique or the vitrification method. The former protocol has been widely adopted in the last years [31], whereas vitrification has been applied more recently [32]. A small

Table 3. Advantages and inconveniences of ovarian tissue freezing

In favor:
– Encouraging results over the last few years
– A large number of primordial follicles can be stored
– Does not require hormonal stimulation and can be performed in a very short time
– Best option for patients who refuse ovarian stimulation and want to avoid exposition to high estradiol levels
Against:
– Cannot be offered to every patient, but only to those with a well-preserved ovarian function (below 38 years of age)
– Risk of reintroduction of malignant cells, even if very rare in breast cancer
– Invasive surgical procedures for both tissue harvesting and later auto-transplantation

piece of the ovarian cortex should always be sent for histopathological evaluation to assess the number and density of primordial follicles, as well as the presence of cancer cells [33]. Once transplanted, the graft's lifespan is strictly time dependent and usually does not last more than 2 years. The thawed ovarian tissue can be reimplanted either into the pelvis (orthotopic transplant), in the forearm, in the abdominal wall, or in other sites (heterotopic transplant). Schröder et al. [34] has proposed an in vitro model for purging cryopreserved ovarian tissue of tumor cells, allowing a safer replacement of ovarian tissue. Donnez et al. [35] proposed 2 different approaches for ovarian tissue transplantation: re-implantation within the atrophic ovaries or in a peritoneal pouch in the lateral pelvic wall. After transplantation, endocrine function resumption and follicle growth takes about 4–5 months. So far, 17 live births have been obtained after orthotopic grafting [36–39], whereas heterotopic transplantation has led only to 1 pregnancy [40], and the in vitro maturation of isolated follicles from thawed ovarian tissue is still far from being applied in a clinical context [41].

Oocyte Harvesting after Ovarian Stimulation

Embryo freezing after oocyte collection has been successfully applied worldwide and remains a valuable option for women with a partner. On the other hand, the cryopreservation of mature or immature oocytes may be the only option for women without partners. The main factor that can influence the outcome of oocytes cryopreservation is their structural complexity, since the subcellular organelles of oocytes are more sensitive to thermal damage than the pre-implantation embryos [42]. Again, 2 freezing protocols are available: slow cooling and vitrification. They differ by cooling temperature curves and cryoprotectant concentrations. A substantial volume of data is available for slow-cooling protocols, showing good results [43–47]. Vitrification is a technique that uses very high concentrations of cryoprotectant and the immediate exposure to ultra-low temperature to transform cells into glass-like solids during freezing [48–50]. Several studies have been conducted to compare vitrification with slow freezing. Smith et al. [51] found that oocyte survival and pregnancy rates were significantly higher following vitrification than after slow freezing. In the study by Grifo et al. [52], oocyte survival rates, fertilization and blastocyst formation did not differ between the 2 groups. Ubaldi et al. [53] performed 104 intracytoplasmic sperm injections after vitrification with an overall pregnancy rate around of 25.0%. Given the improved results obtained with frozen oocytes, the practice committee of American Society of Reproductive Medicine has recently released a statement on these issues [54]. They concluded that despite the limited number of pregnancies obtained, there had been no increase in chromosomal abnormalities or birth defects in children born from these procedures. However, further prospective studies are required to determine the efficacy and safety of long-term oocyte storage [55]. Many concerns

have been raised on the possible negative effects of the elevated estradiol levels during the ovarian stimulation protocols used for oocytes harvesting. Oktay et al. [56] compared letrozole with tamoxifen for ovarian stimulation in patients with breast cancer in a prospective controlled study. In this study it was found that the administration of either drug together with low-dose FSH produced a better oocyte yield than tamoxifen alone. However, letrozole was preferred because it was associated with lower estradiol levels. Further evidence on the safety and success of ovarian stimulation with letrozole and FSH has been recently published [57]. Rates of cancer recurrence in patients undergoing controlled ovarian stimulation were similar to those seen in patients who did not undergo the procedure.

New Venues: In Vitro Maturation of Immature Oocytes, In Vitro Culture and Isolated Follicle Transplantation

As already described, mature oocytes must be harvested after gonadotropin-based ovarian stimulation. This has some disadvantages, particularly when a prompt start of chemotherapy is needed. To avoid treatment delay and to reduce exposure to high estradiol levels, the technique of in vitro maturation (IVM) has been developed. It comprises the pick-up of mature and immature oocytes, which are eventually matured in vitro under controlled conditions [58]. Oocyte collection usually takes place during the mid-follicular phase of the menstrual cycle or during the luteal phase. Immature oocytes are then matured in vitro using medium supplemented with sera, gonadotrophins and estradiol [59]. Data regarding fertilization rates, pregnancy outcomes and live births for cancer patients who have undergone IVM are still limited. The live-birth rate per vitrified oocyte in IVM cycles is about 20%, which is certainly lower than embryo freezing and in vitro fertilization [60]. Although more evidence is required, IVM is an important new fertility preservation strategy for young patients with breast cancer who are not candidates for ovarian stimulation.

Recently, other methods for gamete preservation include in vitro follicle growth (IVG) and isolated follicle transplantation. For IVG, primordial follicles are extracted from cryopreserved tissue and grown into Graafian follicles with specific growth and differentiation factors [61]. The potential for a complete IVG from primordial follicles is realistic, but at the present time this has only been achieved in mouse. Another possibility is the isolation of specifically identified ovarian stem cells before treatment, with subsequent cell implant into the ovary, after chemotherapy has been administered [62].

Conclusions

Young patients are faced with extraordinary burden when a diagnosis of breast cancer is made. Scientific and technological improvements offer a realistic possibility for preserving

fertility even when a high risk of infertility is predicted. Concomitant treatment with GnRHa during chemotherapy, freezing ovarian tissue prior to treatment, and ovarian stimulation with oocytes or embryo freezing are techniques that have been recently developed, and all young patients should receive onco-fertility counseling, with the caveat that most techniques remain experimental. Oncologists should be aware of these possibilities, and establish adequate networks with the reproductive endocrinologists to favor a prompt access to fertility preservation.

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Disclosure Statement

Authors report no conflict of interests.

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